

# CHAPTER 1

## GENERAL INTRODUCTION

### 1.1 Overviews and Objectives of This Thesis

This work investigated modification of polystyrene (PS) culture dish surfaces by means of two types of nitrogen-containing plasma processes. In chapter 2, operated with nitrogen-containing gas plasma modification onto PS dish surfaces. A 13.56 MHz inductively coupled discharge plasma reactor with a mixture of N-containing gas ( $N_2$  or  $NH_3$ ) and noble gas (He or Ar) has been used. This was expected to introduce the N-containing functional groups (e.g.  $-NH_2$ ) and the more hydrophilic groups on PS surface and favored the Wharton's Jelly mesenchymal stem cells (WJMSCs); BCP-K1.

However, a major concern for the practical relevance of a process for the surface functionalization is a durability of product. The process of plasma modification of polymers generally leave the surface in an activated state. Previous studies showed that the concentration of functional groups, introduced on a polymer surface by plasma gas discharge treatments, may change over time, depending on the environment and temperature, especially amine ( $-NH_2$ ) group. In this section, we focused on the long-term effect of physical-chemical properties of treated surface by using the noble gas mixture (Ar or He) on enhancement of reactive species in nitrogen-containing gas plasma. Notwithstanding, the result of this section showed the hydrophobic recovery of treated surfaces over 30 days increases and had trend like untreated PS surface, including this cell culture system had to use animal serum (FBS) condition.

*In vitro* mammalian cell cultures, mammal-derived factors including fetal bovine serum (FBS) are often used as the ideal cell growth supplement and growth factors into the media. FBS comes from the blood drawn from a bovine fetus that causes the concern about the risk of disease transmission such as abnormal prions and various viruses. Therefore, serum- and mammal-free cultures are strongly required. In chapter 3, we focused on sericin hydrolysates that are originated from silkworm. We reported the effect of covalent linkage between a bioactive sericin protein molecule and polystyrene dish surface via a carbon intermediate layer can slow down the released rate of protein into the phosphate buffer saline (PBS) solution. The carbon-coated PS dishes grafted with sericin protein were used in serum-free condition with human bone marrow-derived mesenchymal stem cells (hBM-MSCs).

The chemical inertness of polymers required highly aggressive reactants to incorporate or remove functional groups. This work investigated plasma-chemical approached to create these reactants. A large variety technique of plasma-assisted

processing has been developing that aim at changing the functionalization of a polymer surface. They covered the range from plasma modification to change chemical functional groups over thin films deposition in plasma sputtering system. All employed plasma as an efficient source of highly reactive radicals or other energetic particles to perform or initiated the grafting polymerization process, respectively.

The applicability of plasma processes is not limited to surface. During the last decades, also plasma processing of gases, surfaces and even liquids revealed an enormous technologies and economical potentiality. In polymer industry plasma-based improvement of glueability, printability, metallization and hard coatings are used in mass production, control to cell adhesion by surface functionalization for medical applications and sterilization are becoming a key for medical applications and sterilization that are becoming a key technology for the development of advanced material.

To control a plasma process would mean to influence selected chemical or physical reactions, e.g. to emphasize a specific reaction path. This could be achieved by influencing internal plasma properties and external parameters. In practice, it is hard to control internal plasma properties like the energy distributions of electrons and ions, which dominated the probabilities for overcoming energy thresholds for the generation of reactive species. Another factors is external parameters can be physical, e.g. process pressure, amount and modulation of incident power, ion energy, distance between plasma and substrate, and substrate temperature, or chemical e.g. the choice of gas composition.

Surface functionalization by means of plasma is a complex process. There are three fundamental ingredients that determined the outcome of a plasma treatment. *The plasma chemistry* governs the generation of excited, chemically reactive species in the gas phase. These species may induce surface modifications in what shall be called *plasma surface interactions*. Rearrangements of a modified surface and heterogeneous reactions of activated sites, fragments of the plasma process, give rise to so-called post-plasma surface processes that may drastically change the surface composition, it can be called *hydrophilic/hydrophobic recovery*.

### **Objectives of This Thesis**

The present work was initiated by biomaterial research. N-containing functional groups play a key role in biomaterial application of polymer, especially polystyrene (PS) culture dish.

This work aims to develop the plasma processing modification onto PS culture dish surface by using both of N-containing gas plasma treatment and N-biomolecules immobilization processes. In chapter 2, we investigated the adding/grafting of

oxygen- and nitrogen- functional groups on PS by means of plasma functionalization by PECVD system and to estimate the influence of noble gas to stabilize of treated-functional groups on surface. In particular, these treated-surface PS dishes favored and improved the cell behavior of mesenchymal stem cells.

In chapter 3, we aimed to characterize the immobilization of sericin protein molecules onto PS dish surface via a carbon intermediate layer produced by plasma sputtering system. To demonstrate the covalent binding between sericin onto surface could slow down the release rate of sericin molecules into solution. We finally used the plasma modified-PS culture dishes in serum- and serum-free conditions of mesenchymal stem cells culture.

## 1.2 Theoretical Backgrounds

### 1.2.1 Fundamental of Plasma

‘Plasma’ or called the fourth state of mater, is an ionized gas in which electrically conducting medium comprise of equal numbers of positively and negatively charged particles (Encyclopedia Britannica online).

Plasma states can be divided in two main categories: hot or thermal plasmas (near equilibrium plasmas) and cold or electrical plasmas (non-equilibrium plasmas). Hot plasmas are characterized by very high temperatures of electrons and heavy particles, both charged and neutral and they are close to maximal degrees of ionization (100%). Cold plasmas are characterized by a low temperature of particles (charged and neutral molecular and atomic species), a relatively high temperature of electrons and very low degrees of ionization [1].

Therefore the definition of plasma must be more refined. Francis Chen (1984) proposes three additional requirements to classify plasma [2].

*Quasi-neutrality* is when an equal number of electrons and ions coexist. The implications of this quasi-neutrality can be summarized by the concept of Debye Shielding. When a positive point charge placed in plasma, an electric field is immediately created surrounded by electrons. The electrons form a shield around the positive point charge and at a certain distance, the Debye length ( $\lambda_D$ ), the bulk plasma no longer perceives the point charge. This is true of negative point charges as well, where ions can also form a sheath. For Debye shielding to occur, it is essential that the length of the plasma be greater than the length of the Debye shield. If the length is not greater, then there will be no plasma to be shielded. The plasma profile will be of a charge gradient.

The next necessary characteristic of plasma is *collective behavior*. Collective behavior means that plasma particles are influenced not only by their immediate surroundings, but also by regions a significant distance away. In a medium like air, waves travel only by collisions between one gas molecule and another. However, in plasma if a charge is displaced from its neutral location, it generates an electric field. This electric field disturbs the entire body of charged particles. Each one feels repulsions and attractions resulting in the general motion of all the particles. These moving charges can generate currents, magnetic fields, and influence the behavior of particles a significant distance away.

Lastly, is *motion controlled by electromagnetic forces*. Plasma must have sufficient ionization. Every gas has some fractional ionization, due to the effect of UV radiation, ionizing radiation from natural radioactivity, or the effect of an electric field caused by neighboring power lines, however this ionization is usually small enough to be considered negligible. The acceptable amount of ionization that allows for classification of an ionized gas mix as plasma is a combination of two factors, the collective behavior (the plasma oscillations), and the time between ionized particle and neutral gas collisions. With  $\omega$  defined as the frequency of plasma oscillations, and  $\tau$  as the time between ion/neutral collisions, then the value of  $\omega\tau$  must be greater than or equal to  $\ell$  in order to classify an ionized gas as “plasma”.

Plasma exists throughout nature. They make up nearly 99% of the universe, and can be found in the vast empty space between planets and solar systems. Here on earth they also occur naturally and lightning is the most commonly observed example. The northern lights are also plasma, with their brilliant variation in colors corresponding to different gases and energy levels.

### 1.2.2 Internal Plasma Parameters

Plasmas are characterized by following main parameters:

*Plasma density*: Plasma consists of charged and neutral particles. In the case of cold plasma the electron and ion densities,  $n_e$  and  $n_i$  are assumed to be equal to each other. Typically  $n_e$  values in low pressure cold plasmas are between  $10^9$  and  $10^{12}\text{cm}^{-3}$ . In electrical cold plasmas the degree of ionization is usually low, with  $n_e, n_i \leq 10^{-4} n_g$ .

*Plasma temperature*: The plasma temperature represents the mean translational energy of the particles in the discharge. In the case of cold plasma, electrons are easily accelerated to energies which are sufficient to ionize the gas particles, with typical values of electron temperature,  $T_e$ , in the range of  $10^4$  to  $10^5$  K. These values correspond to  $\sim 0.5 - 10$  eV. The ion temperature,  $T_i$ , is approximately the same as

the room temperature ( $T_{\text{room}}$ ) as well as the neutral particle temperature ( $T_{\text{g}}$ ). So the particles temperatures obey the relation  $T_e \sim T_i \sim T_g \sim T_{\text{room}}$  [3].

*Electron energy distribution:* The electron energy distribution of non-equilibrium low-pressure plasmas can be often described by a Druvesteyn approximation are often not well-described by a Maxwellian distribution function, where the temperature of the electron is considered to be much higher than that of the ion and neutral temperature, and where it is assumed that the energy transfer of electrons is by elastic collisions. Physically, the Druvesteyn distribution is specific to electrons interacting with neutrals of comparatively low energy. Figure 1.1 is a comparison of Maxwell and Druvesteyn electron energy distribution functions at the same value of mean electron energy. The electron energy in the Druvesteyn distribution decreases with energy much faster than the Maxwellian distribution for the same mean energy. It is therefore possible that a Druvesteyn distribution may be found in a low energy plasma. It can be observed that a small number of electrons belong to the low energy electron range (0.5-5 eV). Since the ionization potentials of the atoms of common organic compounds (e.g.  $C^+ = 11.26 \text{ eV}$ ,  $O^+ = 13.6 \text{ eV}$ ,  $N^+ = 14.53 \text{ eV}$ ) belong to the tail region of the electron energy distribution, there is a low degree of ionization in the cold plasmas.

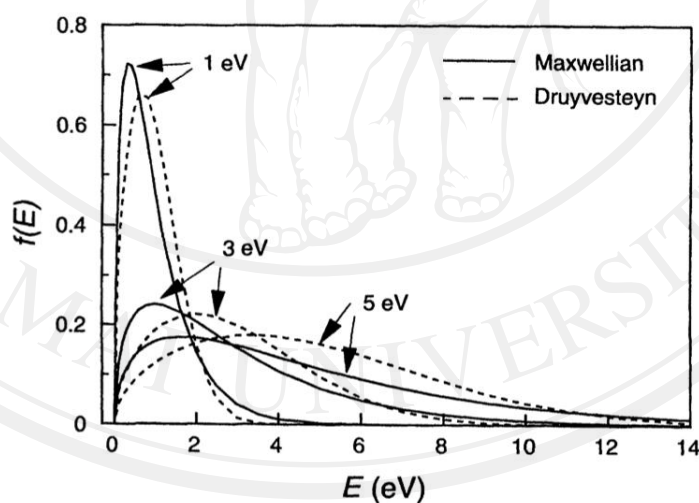


Figure 1.1 Electron energy distribution according to Druvesteyn and Maxwell. The numbers indicate the average electron energy for each distribution [4].

It is important to note that the range of energy of most of the electrons (2 – 5 eV) is high enough to dissociate almost all the chemical bonds involved in organic compounds (Table 1.1), and to create free radical species capable of reorganizing into macromolecular structures. High energies are usually required for the dissociation of unsaturated bonds and the formation of multiple free radicals. Accordingly, original or plasma-generated unsaturated bonds will have a better survival rate under plasma conditions, in comparison to  $\sigma$  bonds.

*Debye length:* The electrical neutrality of a plasma is true only in the macroscopic sense. The electric field of each particle interacts with the electric charges of the surrounding particles. Neutrality of the plasma is achieved when the field of each particle is compensated outside the zone where shielding occurs. The characteristic parameter that describes the electrical shielding (when the potential of each particle is shielded by charges of the surrounding particles) is called the Debye length ( $\lambda_D$ ), and it defines the volume (Debye sphere) within which the neutrality rule can be violated. An ionized gas is considered as a plasma only if the Debye length is much smaller than the physical dimensions of the plasma region.

*Sheath potential:* Charged and neutral particles collide in the plasma phase and with surfaces, which confine the discharge. Due to a higher flux of electrons the surface would gain a negative potential. This results in a positively charged plasma layer of a thickness of several Debye lengths in the vicinity of the surface. This positively charged plasma layer is recognized as the plasma sheath.

Table 1.1 Bond energies and enthalpies of formation of free radicals. [3]

Bond energies		Enthalpies of formation of free radicals	
Species	Energy (eV)	Species	Energy (eV)
C-H	3.3	$\bullet\text{CH}\bullet$	6.1
C-N	7.8	$\text{CH}_2\bullet$	4.4
C-Cl	4.0	$\text{CH}_3\bullet$	1.5
C-F	5.7	$\text{HC}=\text{C}\bullet$	5.8
C=O	11.2	$\text{HC}=\text{CH}_2\bullet$	3.1
C-C	6.3	$\text{NH}\bullet$	3.6
C=C	7.6	$\text{NH}_2\bullet$	1.9
$\text{C}\equiv\text{C}$	10.0	$\bullet\text{Si}\bullet$	4.7
$\text{CH}_3\text{-H}$	4.5	$\text{C}_6\text{H}_5\bullet$	3.4

### 1.2.3 Plasma Chemistry

The electrons, produced in the plasma by the partial ionization of the gas, act as the main agent for the transfer of energy from the external electric field to the gas. The energy transfer is done either by elastic or by inelastic collisions between the electrons and the molecules of the gas. Elastic electron-molecule collisions cause an increase in the kinetic energy of the molecules, while the energy transferred in

inelastic collisions leads to dissociation and ionization of molecules and formation of plasma species such as free radicals, excited metastable, and ions.

The neutral, chemically unstable fragments obtained by the dissociation of the molecules are called radicals. Neutral single atoms, fragments of multiatomic molecules, which are unstable and very active, can also be considered radicals and will be referred to as such. In this section, atoms will be marked as  $A$ ,  $B$ ; molecules as  $M$ ; radicals as  $R$ ; and excited species, at energetic levels above the ground level, with the superscript  $*$ , for example  $R^*$ . Atomic, molecular, or radical positive ions will be marked, respectively,  $A^+$ ,  $M^+$ , and  $R^+$ .

### *Chemical reactions*

The chemical reactions in a plasma reactor can be classified into homogeneous and heterogeneous reactions. The homogeneous reactions occur between species in the gaseous phase as the result of inelastic collisions between electrons and heavy species or collisions between heavy species. Heterogeneous reactions occur between the plasma species and the solid surfaces immersed or in contact with the plasma.

#### *1) Homogeneous reactions*

##### *Reactions of electrons with heavy species:*

The electrons in the plasma gain energy from the external electromagnetic field and transfer it to the gas to excite and sustain the plasma. The major energy transfer from the electrons to the heavy species takes place by inelastic collisions and lead to a variety of reactions such as;

- *Excitation*

Impact of electrons of sufficiency energy with heavy targets leads to the production of excited states of atoms and molecules, as described in the reactions:



The excited states return to ground state emitting their energy as electromagnetic radiation. This radiation accounts for the ultraviolet to visible emissions of the plasma.

- *Dissociative attachment*

When electronegative gases are used, electrons of low energy ( $<1 \text{ eV}$ ) can attach themselves to the molecules of the gas. If such attachment results in the formation of a repulsive electronically excited state, the molecule dissociates very fast ( $\approx 10^{-13} \text{ sec}$ ), producing a negative ion according to equation



Negative ions can also be produced by dissociative ionization reactions:



The reaction described by Eq. (4) is also called dissociative capture, while the reactions described by Eq. (5a) and Eq. (5b) are also called ion-pair formation reactions.

- *Dissociation*

An inelastic collision of an electron with a molecule can cause its dissociation without the formation of ions, according to



The dissociative attachment, dissociative ionization, and dissociation reactions are a major source for the production of atoms, free radicals and negative ions in the cold plasma.

- *Ionization*

Ionization in discharge taking place in a molecular gas occurs predominantly by electron impact that can produce positive or negative, atomic, or molecular ions:



Ionization potentials of some atoms and molecules are shown in Table 1.2. As can be seen in Table 1.2, the ionization potential span the range from 8 to 25 eV, which are above the mean electron energy in a cold plasma (as shown in Figure 1.1).



Therefore, only electrons in the high-energy tail of the electron distribution can contribute to the ionization reactions.

Positive ions are usually formed in most ionization processes prevalent in the cold plasmas. Nevertheless, when the atom or molecule involved in the reaction possess electron affinity, negative ions can also form in the cold plasma, according to Eqs. (4), (5a), and (8b). The process described by Eq. (8b) is a radiative attachment that is very slow and its typical cross section is  $10^{-19} \text{ cm}^2$  at a few electron volts [5].

- *Recombination*

The charged particles (electrons and ions) are lost from the plasma by recombination of particles of opposite charges. Recombinations take place between electrons and atomic ions are accompanied by emission of electromagnetic radiation and are called radiative recombinations:



where  $h$  = Plank's constant

$\nu$  = radiation frequency

The term  $h\nu$  indicates release of radiation energy.

On the other hand, the release of energy during the recombinations of electrons with molecular ions can cause the dissociation of the molecule by a dissociative recombination reaction:



The rates of recombination of electrons with atomic ions, Eq. (9), are very low, in the range of  $10^{-3} \text{ cm}^3 \cdot \text{sec}^{-1}$ . The rates of recombination with molecular ions, Eqs. (10a) and (10b), are much higher, in the range  $10^{-9}$  to  $10^{-10} \text{ cm}^3 \cdot \text{sec}^{-1}$  [6].

Table 1.2 Ionization potentials of atoms and molecules (in electron volts; eV) [5].

Neutral	Ion	Ionization Potential
Ar	Ar <sup>+</sup>	15.8
Ar <sup>+</sup>	Ar <sup>++</sup>	27.6
F	F <sup>+</sup>	17.4
H	H <sup>+</sup>	13.6
He	He <sup>+</sup>	24.6
N	N <sup>+</sup>	14.5
O	O <sup>+</sup>	13.6
Si	Si <sup>+</sup>	8.1
CH <sub>4</sub>	CH <sub>4</sub> <sup>+</sup>	13
C <sub>2</sub> H <sub>2</sub>	C <sub>2</sub> H <sub>2</sub> <sup>+</sup>	11.4
H <sub>2</sub>	H <sub>2</sub> <sup>+</sup>	15.4
HF	HF <sup>+</sup>	17
H <sub>2</sub> O	H <sub>2</sub> O <sup>+</sup>	12.6
N <sub>2</sub>	N <sub>2</sub> <sup>+</sup>	15.6
O <sub>2</sub>	O <sub>2</sub> <sup>+</sup>	12.2
SiH <sub>4</sub>	SiH <sub>4</sub> <sup>+</sup>	12.2

### *Reactions between heavy species*

Reactions between heavy species are those occurring during collisions of molecules, atoms, radicals, and ions. The reactions between the heavy species can be grouped in two subcategories, namely, ion-molecule and radical-molecule reactions. Ion-molecule reactions are those that involve at least one ion. Radical-molecule reactions are the reactions occurring between neutral species only. In a cold plasma, the three densities are related by [7]

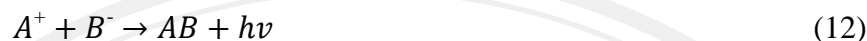
$$n_i \ll n_r \ll n_n \quad (11)$$

where  $n_i$  is the radical density,  $n_i$  is the ion density,  $n_n$  is neutral density.

### *Ion-molecule reactions:*

- *Recombination of ions*

The colliding ions can recombine to form a molecule in the ground state and release their energy through emission of radiation:



A collision between two ions also results in the neutralization of the ions by formation of two excited atoms:



Ion-ion recombination can also take place through a three-body collision:



The two body reaction described by Eq. (12) is important at very low pressures, while three-body recombination occurs at pressure above 0.1 *mTorr*.

- *Charge transfer*

An electric charge may be transferred during a collision between an ion and neutral particle. The transfer can take place between identical partners, for example,



or dissimilar partners:



When the charge transfer takes place with dissociation of the colliding species according to Eq. (16), the reaction is called charge transfer with dissociation:



The reaction rate constants of ion-molecule charge transfer reactions are typically of order of  $10^{-10}$ - $10^{-12}$   $cm^3 \cdot sec^{-1}$  [8].

- *Transfer of heavy reactants*

This type of ion-molecule reaction results in the formation of new compound species as in Eq. (17):



- *Associative detachment*

In a collision between a negative ion and a radical, the ion can attach itself to the radical, neutralizes by releasing the electron, and forms a new compound. This is an associative detachment reaction described by the equation



- *Radical-molecules reactions*

Radical-molecule reactions are those where only neutral species act as reactants. The active radicals may be either multiatom radicals or single atoms, fragments of multiatomic molecules. The radicals are unstable and chemically very active. Representative radical-molecule reactions are presented in the following.

- *Electron transfer*

This is a reaction between two neutral particles resulting in the formation of two ions by the transfer of an electron between the colliding neutrals:



- *Ionization*

A collision between two energetic neutrals can cause the ionization of one of them:



- *Penning ionization/dissociation*

The Penning reactions occur during collisions involving energetic metastable species. In the collisions of metastables ( $B^*$ ) with neutral species, the excited metastables transfer their excess energy to the target, causing ionization or dissociation according to Eq. (21a) or Eq. (21b):



The Penning processes are particularly important in plasma sustained in mixtures, which include gases such as argon and helium, these are characterized by several long-life time metastable states. Furthermore, Penning ionization has a large cross section, which enhances the probability of this process.

- *Attachment of atoms*

These are reactions similar to the associative detachment reactions of the ion-molecule type described by Eq. (22) but involve only neutral species:



- *Disproportionation*

The disproportionation reaction resembles to the ion-molecule transfer of heavy reactants reaction, but it occurs between neutral species according to



- *Recombination of radicals*

Collisions between the chemically active radicals can cause their recombination into stable molecules. The requirement of simultaneous conservation of both the energy and momentum prevents the direct recombination of two monoatomic radicals.

- *Chemiluminescence*

Excitation of an atom or a molecule can take place in the plasma during a collision with another atom. The excitation can occur during a chemical reaction (e.g., disproportionation or recombination), but can also take place without the occurrence of a chemical reaction. The two possibilities are illustrated by Eq. (24) and Eq. (25):



Equation (25) describes a Penning-type reaction. The excited radical  $BC^*$  usually returns from its excited state to its energetic ground level by radiative decays:



Equations 24-26 describe the chemiluminescence reactions that can occur in the plasma and contribute to its luminescence.

## 2) *Heterogeneous reactions*

The heterogeneous reactions are those occur as a result of interactions between a solid surface ( $S$ ) exposed to the plasma and plasma species. The plasma species can be an individual atom ( $A, B$ ), a monomer molecule ( $M$ ), a simple radical ( $R$ ), or a polymer ( $P$ ) formed in the plasma. Typical heterogeneous reactions are presented in:

- *Adsorption*

When molecules, monomers, or radicals from the plasma come in contact with a surface exposed to the plasma they can be adsorbed on the surface. The adsorption reaction can be described by



The indexes  $g$  and  $s$  indicate, respectively, a species in the gas or solid phase. Most radicals interact with surfaces, and consequently the composition of the deposited film will be largely determined by the relative fluxes of film-forming species [9].

- *Recombination or compound formation*

Atoms or radicals from the plasma can react with species already adsorbed on the surface to combine and form compounds according to



$S - A$  indicates an atom  $A$  adsorbed on the surface  $S$ .

During, recombination, the energy of the particles participating in the reaction is usually released as heat to the surface. The rate of surface recombination is strongly dependent on the catalytic properties of the surface.

- *Metastable deexcitation*

An excited metastable species  $M^*$  from the plasma can release its energy and return to the ground state by collision with a solid surface.

The reaction



describes the metastable deexcitation reaction.

- *Sputtering*

A surface exposed to the plasma is always negative relative to the plasma causing positive ions from the plasma to accelerate toward the surface. If an ion  $A^+$  arrives at the surface with sufficient energy, it can remove an atom from the surface:



This process is called sputtering. The atom  $B$  in Eq. (30) can be in this case either an atom from the solid surface or an atom adsorbed on it. The sputtered neutral atom enters the plasma with a kinetic energy of several electron volts.

- *Polymerization*

Radicals in the plasma can react with radicals adsorbed on the surfaces and form polymers:



Polymerization or radical formation can also happen between two species adsorbed on the solid surface:



Polymerization or radical formation can also happen between two species adsorbed on the solid surface:



When a molecule ion collides with a solid surface, it is generally dissociated into its constituent atoms. Upon collision, part of the kinetic energy of the ion is transferred into internal energy, which is generally much larger than the energy required to break the chemical bond. This caused the dissociation of the molecular ion on impact on the solid surface. For example, the energy required to break the bond between the atoms of the nitrogen molecule is about  $9 \text{ eV}$ . It was found that the probability of breaking up an  $N_2^+$  ion on collision with a solid surface has a threshold of around  $9 \text{ eV}$  and reaches 100% at  $\sim 100 \text{ eV}$ .

Negative ions are usually ignored in the mechanistic discussions of plasma processing of surfaces. Because any surface in contact with a plasma is at a negative potential relative to the plasma, the negative ions created in the plasma cannot reach the surface and are usually ignored in the discussion of plasma interaction with surfaces. However, in some situations negative ions will participate in surface reactions. Such situations arise whenever a negatively biased surface has a tendency to form negative secondary ions when bombarded with positive ions. These negative ions will be accelerated into the plasma by the sheath potential, and if the operating pressure and interelectrode distance are sufficiently low, the negative ions can traverse the plasma arriving at the opposite electrode with substantial kinetic energy.

This effect has been observed during sputter deposition of halides and plasma deposition of high-temperature superconducting materials [10].

*The chemical reaction occurring in the plasma bulk or at the surface in contact with plasma:*

Chemical reactions that take place at a surface exposed to the plasma can be affected by ultraviolet photons and soft X ray present in the plasma that are sufficiently energetic to break chemical bonds. However, electron and especially ion bombardments are the most effective in changing and promoting chemical reactions at the substrate. In addition to physical sputtering, ion bombardment promotes the mixing of atoms near the surface by momentum transfer and by enhanced diffusion. This bombardment is partially responsible for the improvement in the quality of thin films that are deposited with simultaneous energetic ion bombardment. Electron bombardment was also found to cause an enhancing etching effect. Because the momentum of ions is much higher than that of electrons, the ion bombardment is much more effective in enhancing surface reactions than is electron bombardment.

Another aspect of the plasma-surface interactions is the transfer of energy. Energy transfer from plasma to solid surfaces occurs through optical radiation and fluxes of neutral particles and ions. The optical radiation has components in the infrared, visible ultraviolet, and sometimes soft X ray. When absorbed by a solid surface, the radiation usually transforms into heat. When the surface is a polymer, absorption of ultraviolet radiation can also break up the polymer to produce free radicals that can react with arriving plasma species.

The energy of the neutral particles is composed of kinetic, vibrational, dissociation (for free radicals), and excitation (for metastables) fractions. The dissipation of the kinetic and vibrational energy fractions causes heating of the substrate. The dissociation energy can also be dissipated through surface chemical reactions on polymers or through surface reactions involving adsorbed species and recombinations on metals. The metastable species release their energy only through collisions that cause heating of metallic surfaces or the formation of free radicals on polymers [11].

The energy of the ion flux is composed of kinetic, vibrational and electronic fractions. The ions are accelerated toward the surfaces exposed to the plasma that are negatively self- or externally biased relative to the plasma. The ion bombardment can affect the properties of the deposited film if the ion energy is sufficiently high. In RF plasma, the kinetic energy with which an ion reaches the electrode depends on the ratio between the distance passed by the ion during half a cycle and the width of the plasma sheath. This distance is a function of the ion mobility, the sheath field, and the frequency of the electromagnetic field sustaining the plasma. The ionization



energy is released by neutralization and is dissipated by either heating of metals surface or formation of free radicals polymers.

Ion impact that occurs on the negatively biased surfaces can affect the physical properties of the material. During deposition, it can cause densification and increased oxidation resistance of the films. Ion impact in PECVD can also cause enhanced diffusion, collisional mixing, and the formation of metastable materials, such as amorphous hydrogenated silicon or amorphous hydrogenated carbon (diamond like carbon).

However, the bombardment of the substrate with high energetic particles or the large fluxes damage in existing films or substrate. The damage is caused through bond breaking by the bombarding particles and formation of defects in the surface layer of the substrate.

During ion bombardment, impurities from the plasma can penetrate the substrate either by implantation or through enhanced diffusion. Hydrogen atoms are especially prone to penetrate the substrate because of their small size. Ion bombardment can cause formation of point defects in the crystal lattice. The plasma can also cause contamination of the substrate with material sputtered from the walls of the reactor or the electrodes. The degree of damage can be reduced by decreasing the exposure time to the hydrogen-containing plasma.

### **1.3 Plasma Nanotechnology**

Plasma is a quasineutral gas of charged and neutral particles which exhibits collective behavior. Plasma surface modification can be used to modify a very thin surface layer (in *nm* to  $\mu\text{m}$  scales) of a polymer without altering its bulk characteristics. In addition, plasma treatments can involve chemical modification of the surface, etching of the surface materials, or plasma polymerization, in which a plasma polymer is deposited on the surface.

High-tech plasma processing technology is indispensable for the development and application of highly functionalized new nano-materials and nano-devices. "Plasma-Nano science" is interdisciplinary research area that is based on plasma science, electronics, applied physics, applied chemistry and process engineering. Recently, plasma processing became an attractive medium for synthesis and modification of nanoparticles such as carbon nanotubes inorganic nanowire and others.

The nanotechnology research community recognized the versatility and unique advantages of various discharges and adopted the technique to grow nanostructured

materials and to a limited extent, for further processing of these materials. From the reviewed, most of these attempts are in their early stages, except perhaps in the case of PECVD of CNFs and nanotubes, which has been studied more extensively. Preliminary results in each of the areas are strongly encouraging and the anticipation is that the utility of low-temperature plasmas in nanomaterial preparation will see a significant growth [12].

#### 1.4 Plasma Surface Modification Process

Plasma is created under energy deposition into gaseous mixture, mainly generated by electric field transmits energy to the neutral species by collisions to produce excited species and ions. The electric field transmits energy to the gas electrons. Gas turns into plasma due to ionization, dissociation and excitation of the bounded states of atoms and molecules of the background gas. Plasma consists of gaseous mixture of charged particles (free electrons, ions) and neutral activated species including gas molecules, radicals, metastables and UV-photons, as shown in Figure 1.2.

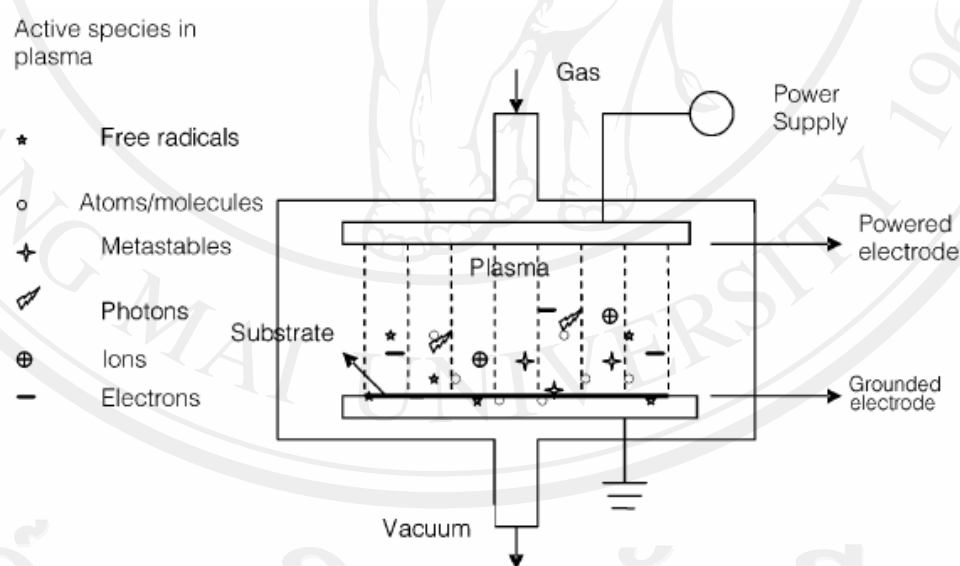


Figure 1.2 Schematic diagram of cold plasma reactor [13].

Plasma surface treatment is the use of plasma species produced under plasma environment to modify the surface characteristics of solid materials. Low-pressure plasma, cold plasma, nonequilibrium plasma and glow discharge are some of the synonymously used terms to designate the same type of process. When used plasma to treat polymers, the plasma species have sufficiently high level of energy to break the covalent bonds of polymers created free radicals (or active sites) and there are

coupled with active species from the plasma environment. Plasma surface modification treatments of polymers are aimed to bring about surface functionalization, which creates new surface functional groups and thus new surface properties. Generally, plasma discharges can chemically modify a polymer surface by surface functionalization using reactive gas plasma produced new surface functional groups [14, 15], or by surface cross-linking via activated species of inert gases plasma such as argon or helium [16-18]. The reactive plasma of some gases (such as O<sub>2</sub>, N<sub>2</sub>, NH<sub>3</sub>, CF<sub>4</sub>, H<sub>2</sub>O, CO<sub>2</sub>, air, etc.) can result in the incorporation of some of the species of the gas into the surface functionalities of the polymer [19, 20].

The interaction of plasma with substrate occurs, when the reactive plasma species (photons, positive ions, high-energy electrons, free radicals, electronically excited molecular and atomic species) are generated ionization, fragmentation, and excitation. When a polymer is placed in plasma surroundings, a plasma sheath is formed around the surface that accelerates nearby ions. Some electrons that contain high enough kinetic energy can overcome the sheath potential and impact the polymeric surface. These charged species bombard the surface with high energy that allows them to react with the surface macromolecules or penetrate the surface and transfer their energy to the polymer. Energy transfer could also result from diffusion of metastable neutral species and irradiation by photons that absorb into the polymer surface layers and can cause hydrogen abstraction or ablation of side-group species or chain scission on polymer chain, depending on the energy levels and the polymer structure.

The possible reaction pathways that can occur at the plasma-polymer interface during plasma treatments of polymers may be categorized as: (i) surface modification [21-23], (ii) grafting [24-25] and (iii) film deposition [26, 27].

Figure 1.3 illustrates the interactions of the various plasma species with the polymer surface.

A list of gases used in plasma processing, including polymerization is provided Table 1.3.

In this process, gases or monomers in the plasma undergo polymerization through a free-radical initiation process. When sufficient energy is supplied to break all the bonds of the process gas mixture such as methane, ethylene, propylene, fluorocarbon monomers and organosilicon compounds, these molecules are fractured into free-radical fragments and start to initiate polymerization deposited on the surface of the substrate. The plasma-polymerized thin films are generally pinhole-free, highly cross-linked and strongly bound to the surface.

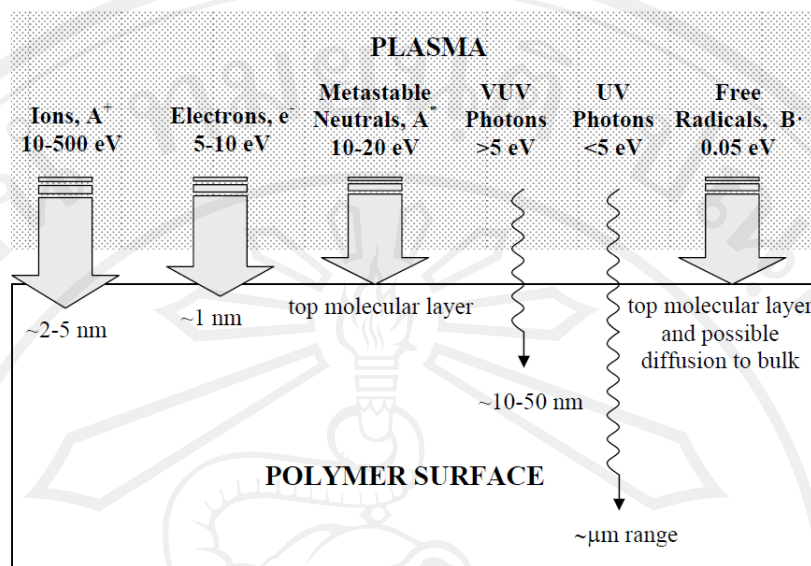


Figure 1.3 Schematic illustrations of the interactions of the plasma phase species with the polymer surface (summarized from references 28-29).

Table 1.3 Plasma gases and their applications. [30]

Plasma gas	Application
Oxidizing gases (O <sub>2</sub> , air, H <sub>2</sub> O, N <sub>2</sub> O)	Removal of organics by oxidation and to leave oxygen species in the polymer surface
Reducing gases (H <sub>2</sub> , mixtures of H <sub>2</sub> )	Replacement of F or O in surfaces, removal of oxidation-sensitive materials, conversion of contaminants to low molecular weight species that do not polymerize or re-deposit on adjacent surfaces
Noble gases (Ar, He)	To generate free radicals in surfaces to cause cross-linking or to generate active sites for further reaction
Active gases (NH <sub>3</sub> )	To generate amino groups
Fluorinated gases (CF <sub>4</sub> , SF <sub>6</sub> and other perfluorinated gases)	To make the surface inert and hydrophobic
Polymerizing gases (monomer gases for direct polymerization, Ar or He pretreated)	Polymerization of layers onto substrates by direct polymerization or by grafting on Ar or He pretreated polymer surface

Effect of plasma on a material surface is achieved using gases such as air, O<sub>2</sub>, N<sub>2</sub>, argon and helium, these factors lead to removed of surface contaminants and weakly bound polymer layers, etching and substitution of chemical groups on the surface that allow covalent bonding that contribute to improve adhesion. The removal contaminations is due to simple plasma sputtering with the help of noble gases, oxidation or reduction of organic contaminants with oxygen plasma or reduction of oxides, sulphides by hydrogen plasma. It enhances adhesion by allowing interlinking or covalent bonding of molecules on the surface. The choice of gas used for contaminant removal depends on the nature of the contaminant and the substrate surface.

Plasma etching is a key to removal of material from surfaces which done by selectively removed by chemical reaction and/or physical sputtering. During plasma etching, surface cleaning is done by etching based on a combination of chemical reactions of surface impurities and active radicals to form by-products like CO<sub>2</sub>, H<sub>2</sub>O and low molecular weight hydrocarbons, which are later removed by means of vacuum, and sputtering through ion bombardment. Roughening the surface by plasma etching increases the area of contact.

Substitution of chemical groups into polymer chain can be used different process gases incorporated large varieties of functional groups such as hydroxyl, carbonyl, carboxylic, amino or peroxy groups. The plasma induced chemical processes for example oxidation, nitration, hydrolization and amination are used to improve the surface energy and reactivity. Substituting the polar functional groups increases the surface energy and reactivity and also allows a liquid to spread over, penetrate the surface and form the strong bond between the substrate and the adhesive. This enhances adhesion strength by promoting covalent bonding between the adhesive and the substrate surface. The gases used to generate plasma modified are reactive, unlike in plasma-induced grafting.

Plasma polymerization is essential a PECVD process resulting in the deposition of an organic polymer films on the surface of the substrate. The deposited films are called plasma polymers and are generally chemically and physically different from conventional polymers.

Nevertheless, there are two major path can be considered as plasma surface process of substrate modification, plasma-enhanced chemical vapor deposition (PECVD) system and physical-assisted physical vapor deposition or sputter deposition system.

#### 1.4.1 Plasma-enhanced Chemical Vapor Deposition (PECVD)

PECVD is an excellent alternative for depositing a variety of thin films at lower temperatures which uses electrical energy transferred in to a gas mixture to generate a glow discharge (plasma). Glow discharge plasma consist reactive radicals, ions, neutral atoms and molecules, and other highly excited species. These atomic and molecular fragments interact with a substrate and produce the thin films on surface [31]. Plasma polymerization is essential a PECVD process as mentioned above. The materials formed by plasma polymerization are very different from conventional polymers. Polymers formed by plasma polymerization are an atomic process, used free radical formed on the surface of polymers to initiate graft polymerization, in contrast to conventional polymerization is based on molecular processes. Polymers formed by plasma polymerization are in most cases highly branched and highly cross-linked. The properties of the plasma polymer are not determined by the used monomer but by the plasma parameters.

Plasma polymerization take place through several reactions steps, that is, initiation, termination and reinitiation. In the initiation stage, the energetic electrons and ions collide with monomer molecules create either free radicals or atoms and are adsorbed on the surface when it exposed to the plasma. The propagation of the reactions is the formation of the polymeric chain, can take place both in the gas phase and on the deposited polymer film. In the gas phase, propagation involves the addition of a radical atom to another radical or molecule. At the surface of the polymer film propagation occurs through interactions of surface free radicals with either gas phase or adsorbed monomers. Termination also takes place either in the gas phase or at the surface by processes similar to the propagation step but ending either with the final product or a closed polymer chain.

In plasma polymerization, neutral products formed in the termination step can undergo reinitiation and propagation reactions, while in conventional polymerization the termination step interrupts the process. The monomer in plasma polymerization does not need to have polymerizable groups. Plasma polymerization is used to prepare films for corrosion protection of metals or water barriers for optical elements sensitive to humidity due to the possibility to prepare highly cross-linked, pinhole free films. This process is also used for preparation of gas separation membranes, reverse osmosis membranes for desalinization, abrasive-resistant coatings on softer materials, deposition of dry developable resist for lithography, and dielectric materials [14, 16]. Another example of the applications of plasma polymerization is used for bio applications such as to modify surface of biomaterials to improve biocompatibility property [21, 26, 31-33].

Another PECVD coating is deposition of inorganic coating such as silicon dioxide ( $\text{SiO}_2$ ) films and diamond like carbon (DLC) films.  $\text{SiO}_2$  deposited by

PECVD is used as an interlayer dielectric film between metallization layers in very-large-scale integration (VLSI). Silicon dioxide films have been deposited from a variety of gas mixtures using different plasmas and process parameters. The main precursor is the mixture of silane, nitrous oxide and oxygen, for example tetraethoxysilane (TEOS) and oxygen, nitrous oxide, or ozone, at a variety of frequencies, from 50 kHz to 2.45 GHz. The quality of the films deposited from TEOS and oxygen has also been found to depend on the excitation frequency. Better-quality films were obtained at lower frequencies (150 kHz) than at high frequency (14 MHz). Silicon dioxide has been directionally in a 13.56 MHz plasma from mixtures of oxygen and tetraethoxysilane or octamethylcyclotetrasiloxane (OMCTS). The liquid TEOS or OMCTS were carried into the reactor by helium flowing through the evaporator. The growth directionality was found to be a function of the O<sub>2</sub>:TEOS or OMCTS ratio.

Diamond like carbon or DLC films is metastable amorphous material, which may include a microcrystalline phase. The deposition of DLC films have been prepared by a variety of methods, including DC or RF plasma assisted CVD, sputtering, and ion beam deposition, using different carbon bearing, solid or gaseous, source materials. Any hydrocarbon with sufficient vapor pressure can be used as the precursor for PECVD of DLC films for example acetylene, benzene, butane, cyclohexane, ethane, ethylene, hexane, isopropane, methane [34, 35].

#### **1.4.1.1 Plasma-based Functionalization with Nitrogen-containing Groups**

When used reactive gas such as oxygen- and nitrogen-containing gas plasma to treat polymers, the plasma species have sufficiently high level of energy to break the covalent bonds of polymers created free radicals (or active sites) and there are coupled with active species from the plasma environment, as shown in Figure 1.4 and 1.5. Figure 1.6 shows nitrogen-containing plasma surface modification treatments of polymers are aimed to bring about surface functionalization by nitrogen-containing species, which creates new surface nitrogen functional groups and thus new surface properties.

Plasma functionalization surface technique with nitrogen-containing groups can be categories into two types; (i) using nitrogen-containing gas as precursor and (ii) using nitrogen-containing biomolecules (protein) as immobilized molecule.

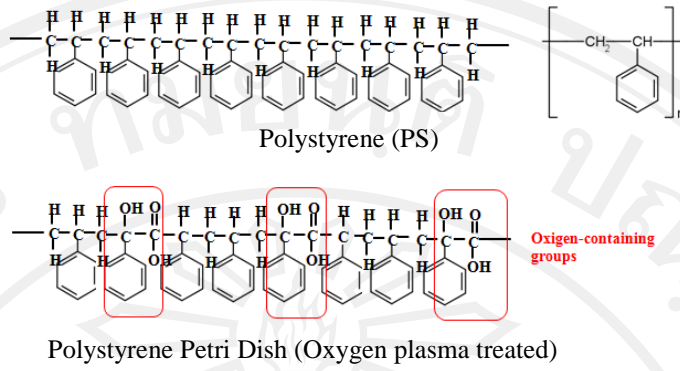


Figure 1.4 Polystyrene structure and polystyrene petri dish structure.

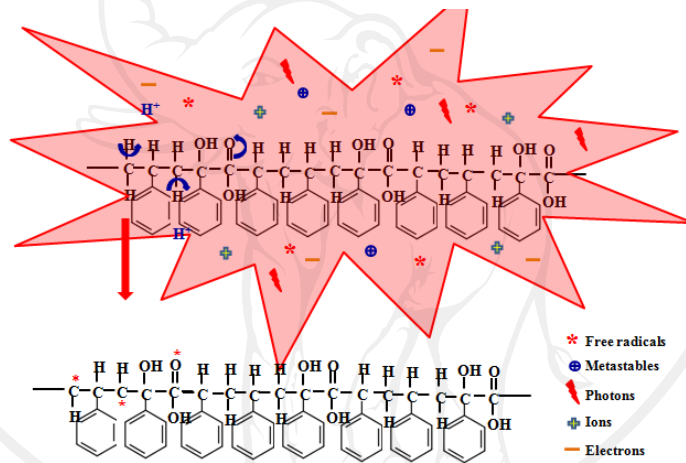


Figure 1.5 Interaction between active sites at polymer surface and plasma species.

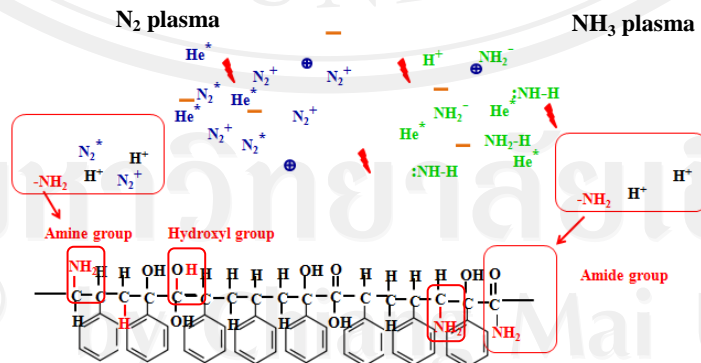


Figure 1.6 Interaction between nitrogen-containing species to create nitrogen-containing functional groups on PS surface in plasma environment.



### 1) Nitrogen-containing Gas Plasma

#### Gas Phase Reactions in Nitrogen-containing Discharges

Amination of polymers can in principle be achieved in many types of discharges and gas mixtures, provided they contain nitrogen. The outcome of the process essentially depends on the composition of the gas phase. Various transient species containing nitrogen and/or hydrogen are candidates for grafting or removing of nitrogen functional groups.

- *Discharges in Nitrogen*

Much of the complexity of discharges in nitrogen must be attributed to the fact that nitrogen is a trivalent molecule. There are no fewer than nine electronic nitrogen states lying below dissociation. A second consequence is that nitrogen can form strong multiple bonds (dissociation energy of  $N \equiv N$  9.8 eV) [36], so that reactions in which these bonds are formed are usually very exothermic and may also be quite fast. Single bonds formed by nitrogen, on the other hand, are not strong. The strong triple bond is also responsible for an often rich and high energetic vibrational spectrum of  $N_2$  in plasma conditions. Vibrationally excited  $N_2$  can substantially influence the plasma chemistry, either by its enhanced reactivity that may lead to dissociation of other molecules.

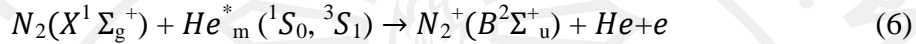
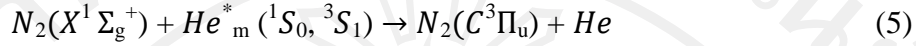
Discharges in pure  $N_2$  have been studied for nitriding purposes of various materials. However, the amount of primary amino groups created on the surface was very low. It could be shown that hydrogen has to be added to the gas phase to achieve satisfactory results. This can either be achieved by adding  $H_2$  or by replacing  $N_2$  by  $NH_3$ . Both gas mixtures were studied and compared in the scope of this work.

The production of nitrogen active species significantly depends on the discharge parameters. Dominant excited species in  $N_2$  plasma are  $N_2^*$  and  $N_2^+$  [37]. In the  $N_2$  plasma, the primary reaction is the ground state  $N_2^+(X^2\Sigma_g^+)$  to the upper state of  $N_2^+$  which mainly by electron collision. The detailed reactions are given below:



In helium-nitrogen mixture plasma, helium has higher metastable energies than any other inert gas meanwhile has also lower efficiency of cathode sputtering owing to its low mass compared to other inert gases. Thus the helium metastable states ( $^3S_1, ^1S_0$ ) having the energies (19.83 and 20.61 eV), higher than the threshold

ionization energy of nitrogen molecule (15.57 eV) can certainly ionize it to produce an electron ion pair [37]. He can be added in nitrogen plasma for enhancing the production of active species without causing any considerable increase in the impurity level. The  $N_2(C^3\Pi_u)$  state can also be populated by utilizing the internal energy of helium metastable states by Penning effect [38, 39]:



By convention the ground state molecular term symbol is prefixed with  $X$ , the first excited state of the same spin multiplicity is  $A$ , the second with  $B$ , and the third with  $C$ .

- *Discharges in Ammonia*

Discharges in ammonia are of special interest for surface nitriding applications since the dissociation energy of  $NH_3$  is considerably lower than that of  $N_2$  (dissociation energy of  $HN-H$  2.0 eV,  $HN-H_2$  4.7 eV,  $H_2N-H$  4.3 eV,  $H_2N-NH_2$  1.7 eV) [40-42]. For similar power input, a discharge in  $NH_3$  may provide higher densities of atomic nitrogen than in  $N_2$ . Incomplete decomposition or reactions with hydrogen may give rise to the presence of  $:NH$  or  $NH_2$  in the discharge. So, an increase of the density of atomic nitrogen over that in pure  $N_2$  is no guaranteed. However, the products  $:NH$  or  $NH_2$  may be efficient in grafting nitrogen functionalities as well.

Generally speaking the study of active species in ammonia plasma is molecular species, during the last decades many authors reported the decomposition of  $NH_3$  plasma [43, 44]. Electron and heavy particle collisions in plasmas are not the only mechanism of  $NH_3$  decomposition. Photodissociation by VUV/UV radiation opens another important channel. Since hydrogen-containing plasmas are efficient in generating VUV/UV radiation.

It is known that, under discharge conditions, ammonia and its fragment species can undergo the primary decomposition process which is generally favored:



The  $NH$  radical emission spectrum has also been observed, but the radical has generally been considered of minor importance and probably formed by the decomposition of  $NH_2$ :



A major role for  $NH$  has been postulated in the photochemical decomposition of  $NH_3$  in the vacuum:



A variety of radical reactions that have been proposed for the primary decomposition processes in RF discharges. These conclude



A plasma in gases containing nitrogen and ammonia produces a large number of transient species. Some of them have sufficient reactivity to modify polymer surfaces. Among them are VUV/UV photons,  $NH, NH_2, H, N$  radicals, ions, metastables of  $NH, N_2, N$  and other vibronically excited species. Especially the radicals, ions and metastables deserve our special interest since they are candidates for grafting nitrogen-containing surface functionalities. But also surface activating processes of photons may enhance the surface reactivity of low energetic chemical species. Since molecular and atomic nitrogen have many energetic excitations, traditionally, it was spoken of active nitrogen to denote reactive nitrogen plasma species. Unfortunately, even nowadays, this expression appears appropriate in context of surface functionalization since the individual reaction mechanisms of the different nitrogen-grafting species are still not well understood.

Nitrogen-containing gas plasmas require no wet-chemical processing, there are simple one-step processes a dry plasma treatment process with the groups of nitrogen, ammonia or dried air. This process does not require the use of chemical linker molecules and associated wet chemistry, it results in covalent attachment between the amine and the polymer surface as well as preservation of the bioactivity of the treated surface which is greatly improved over that achievable by simple physical adsorption.

The general nitrogen-containing gas were used generated nitrogen-plasma include nitrogen ( $N_2$ ), ammonia ( $NH_3$ ) and air. A variety of functional groups may be

incorporated onto the polymer surface, for example, amine ( $-NH_2$ ) and hydroxyl ( $-OH$ ) [35, 45], which are known to be important for the attachment of many different kinds of proteins and cells.

The incorporated Nitrogen-containing groups are capable of efficient interaction with proteins by hydrogen bonding, which might affect the adsorption of serum adhesive glycoproteins. In addition, the positive charged groups ( $-NH_2$ ) also enhance the interaction between the surface of materials and the cells that carry a negative charge.

#### 1.4.2 Physical-assisted Physical Vapor Deposition or Sputter Deposition System

Sputter deposition is a physical vapor deposition (PVD) method of deposition thin films by sputtering. The commonly sputter coatings is hydrogen-free DLC films or unhydrogenated amorphous carbon films (a-C). Amorphous carbon is an attractive coating material in terms of hardness, lubricity, and were resistance. In addition, carbon materials appear more attractive for protein adsorption due to their inherent stability, biocompatibility and surface properties such as porous structure and high surface area. It can also lead to an enhancement of specific binding with a given biomolecule.

The deposition of a-C films necessitates source for the carbon species. The carbon source can be either a carbon-containing gas ionized by an excitation source (consecutive mass section of the  $C^+$  species is then needed) or a pure carbon target (or graphite,  $C_{60}$ ) thermally evaporated, ion sputtered or laser ablated. The energy source can be electrostatic acceleration or momentum transfer by collisions with energetic species. The momentum transfer can occur before deposition (sputtering source) or after deposition (bombardment by energetic species) [46, 47]. The microscopic and macroscopic properties of amorphous carbon (a-C) films depend strongly on the ration between the fraction of  $sp^2$  and  $sp^3$  bonds. Recently, hydrogen-free amorphous carbon films produced from carbon atoms with energies around 30 eV have been shown to contain more than 80%  $sp^3$  bond fraction [48]. In sputtering deposition, one can achieve the appropriate value of compressive stresses by increasing the incoming carbon atoms kinetic energies and their charge states in the plasma. However, it is expected that there is a narrow region of deposition conditions to provide these values where film rich in  $sp^3$  C-C bonds can be formed.

##### *Principle and Process of Sputtering*

Sputter deposition is a physical vapor deposition process for depositing thin films, sputtering means ejecting material from a target and depositing it on a substrate such as a silicon wafer. The target is the source material. Substrates are placed in a

vacuum chamber and are pumped down to a prescribed process pressure. Sputtering starts when a negative charge is applied to the target material causing the glow discharge plasma. Positive charged gas ions generated in the plasma region are attracted to the negatively biased target plate at a very high speed. This collision creates a momentum transfer and ejects atomic size particles from the target. These particles are deposited as a thin film into the surface of the substrates.

Typically, a substrate is placed in a vacuum chamber opposite a sputtered target. The chamber is evacuated and then backfilled with a process gas (Argon) and produced the ionized gas in a glow discharge plasma. A target (or cathode) plate is bombarded by energetic ions as a result of atoms/molecules of target material are physically removed i.e., “sputtering” from the target and landed on the substrate. The intent for this material to arrive at the substrate with enough energy to form a thin, strongly attached film, one monolayer at a time [49, 50]. The basic sputtering process has been known for many years and many materials have been successfully deposited using this technique [51, 52]. However, the process is limited by low deposition rates, low ionization efficiencies in the plasma, and high substrate heating effects. These limitations have been overcome by the development of magnetron sputtering and, more recently, unbalanced magnetron sputtering.

Magnetron sputtering is one of several types of sputtering. Magnetron sputtering can be done either in DC or RF mode. DC sputtering is done with conducting materials. If the target is a non conducting material the positive charge will build up on the material and it will stop sputtering. RF sputtering can be done both conducting and non conducting materials. Here, magnets are used to increase the percentage of electrons that take part in ionization of events and thereby increase the probability of electrons striking the Argon atoms, increase the length of the electron path (mean free path), and hence increase the ionization efficiency significantly.

Sputtering is extensively used in the semiconductor industry to deposit thin films of various materials in integrated circuits processing. Thin antireflection coating on glass, which is useful for optical application is also deposited by sputtering. Because of the low substrate temperatures used, sputtering is an ideal method to deposit contact metals for thin-film sensors, photovoltaic thin films (solar cells), metal cantilevers and interconnects etc.

#### **1.4.2.1 Nitrogen-biomolecule Immobilization**

##### *Immobilization of Biomolecule in Plasma Polymer*

Development of low-cost, one-step immobilization technology for covalent attachment of biomolecules to polymer surfaces achieved to overcome the drawback

of aging effect of plasma gas surface functionalization. Covalent attachment of biomolecules to polymer surfaces can be achieved using a variety of techniques including wet chemical treatments, silane monolayers, plasma and UV irradiation as a pretreatment step to render the polymer surface to create a wide range of active functional groups [53-55].

Plasma-induced grafting is a two-step process of incorporation of functional groups and reactive sites to the polymer surface. First, free-radical formation using noble gas plasma is created on surface and followed by the introduction of an unsaturated monomer such as allyl alcohol into the reaction chamber. The monomer reacts with the free radical to yield a grafted polymer on surface. This process differs from plasma polymerization in which the plasma gas itself is a monomer [25].

Plasma surface treatment represents an important step before biomolecules immobilization. After plasma treatment, the polymer surface created active sites and followed by biomolecules immobilization can lead to the covalent attachment between biomolecules and surface. Plasma treatment is one of the efficient methods in the field of surface modification. Compared to chemical modification techniques, plasma treatment has the advantages of shorter reaction time, non polluting processing, while providing a wide range of different functional groups which might be further used to connect and deliver the therapeutic agents in a biomedical application. Among various functional groups, the primary amines (in protein molecules) are the most desirable reactive functionalities for carbon structure. Their presence induces a hydrophilic behavior and reactivity as well. The introduction of amino groups to the polymer surface achieves enhanced wettability and improved adhesion.

The use of plasma sputtering to create a functionalized-amorphous carbon layer on the polymer surface to introduced desired functional groups and plasma grafting methods have been a preferred way for covalent biomolecules immobilization. Many research reported the advantages of the carbon film including a high specific-area, porous structure is a more binding active molecule and biocompatibility which preferred to protein molecule adsorption [56-58].

### **1.5 Plasma Surface Modification Effects on Polymeric Biomaterials**

Biomaterial is any matter, surface, or construct that interacts with biological systems. Biomaterials are thus comprises whole or part of living structure. So, biomaterials have to be biocompatibility, is the ability of material to perform with an appropriate host response in a specific application [59]. In all case, biomaterials contact biological environments that contain biomolecules, such as proteins or living

cells, and the surface of the materials must have qualities that make them compatible with live environment.

The interaction of the biomaterial with living cells or proteins might have to be modified, either to reduce or to enhance that interaction according to the application. Such modifications can be obtained by plasma treatments. These interactions are affected by the biomaterials surface properties, such as [60]:

1. Chemical composition (polar-apolar, acid-base, H-bonding, ionic charges)
2. Surface microstructure (polymer chain ends, loops and their flexibility)
3. Topography (roughness, porosity, imperfections, gas microbubbles)
4. Domains (distribution of any of the foregoing in the surface)

The objectives of plasma surface modification in biomedical applications aim to improve biocompatibility/blood compatibility of any materials which used in biological system. We focused in cell therapy, using mesenchymal stem cells (MSCs), can be used to treat many human diseases. This can be achieved by the transplantation of cells, in sufficiently high numbers and quality, to a target damaged organ. Thus in *In vitro*, the cells must then survive long enough to restore normal function. In the cell culture substrates, plasma process enhances cell adhesion promotion, enhanced surface wettability and spreading, and reduced surface friction. When polymer exposed to plasma environment, plasma active species have sufficiently high energy to break the covalent bonds of polymers surface to make it suitable for specific application. A variety of plasma treatments have been used to provide biomaterials with reactive groups for subsequent bonding of biomolecules. The capability of plasma to modify surface physical and chemical properties without affecting bulk properties is advantageous for the design, development and manufacture of biocompatible polymers. A list of plasma treatment of biomaterial polymers for different applications is presented in Table 1.4.

Biomaterial research developed aims at controlling the adhesion behavior of selected cell types. In biological systems, amino groups are of special importance. In cell culture experiments that were performed in the frame of this work, high densities of biological cells resulted on amino-functionalized polystyrene petri dishes.

Table 1.4 Biomedical applications of cold plasmas. [61]

---

<b>A. Plasma Treatment (Etching)</b>
1. Clean
2. Sterilize
3. Cross-link surface molecules
<b>B. Plasma Treatment (Etching) and Plasma Polymerization (Deposition)</b>
1. Foam barrier film:
a. Protective coating
b. Insulating coating
c. Reduce absorption from environment
d. Reduce release rate of leachables
e. Control drug delivery rate
2. Modify protein and cell interaction:
a. Improve “biocompatibility”
b. Promote selective protein adsorption
c. Enhance cell adhesion
d. Improve cell culture surfaces
e. Provide nonfouling surfaces
f. Reduce surface friction
3. Provide reactive sites:
a. For grafting or polymerizing polymers
b. For immobilizing biomolecules

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In addition to cell-binding peptides, a variety of other biologically active molecules have been used to enhance cell adhesion to surfaces. For certain cells, adhesion has been enhanced by adsorption of homopolymers of basic amino acids, such as polylysine and polyornithine or bioactive molecules. Likewise, covalently bound amine groups have influenced cell attachment and growth [62, 63]. The immobilization method of bioactive molecules onto substrate surfaces is achieved by plasma grafting process. The reactive sites formed in a polymer during its irradiation (e.g. irradiation by ions, plasma or ultraviolet light), such as radicals, conjugated double bonds between carbon atoms and oxygen-containing groups, can be used for grafting various molecules and nanoparticles which further modulate the effects of polymer irradiation on cell adhesion, growth, phenotypic maturation and functioning. These biomolecules and nanoparticles include glycine, alanine and leucine, RGD-



containing adhesion oligopeptides, bovine serum albumin, polyethylene glycol, colloidal carbon particles and gold nanoparticles [64, 65].

Surface modification techniques have been used to produce polymers for cell attachment. For example, chemical groups can be added to change the wettability of the surface, which often influences cell adhesion. Alternatively, whole proteins such as collagen can be immobilized to the surface, providing the cell with a substrate that more closely resembles the ECM found in tissues [66]. Collagen and other ECM molecules have also been incorporated into hydrogels by either adding the protein to a reaction mixture containing monomers and initiating polymerization [67], or mixing the protein with polymerized polymer.

At present study, reported that ECM proteins are commonly used in cell culture systems to maintain stem and precursor cells in undifferentiated state during cell culture and function to induce differentiation of epithelial, endothelial and smooth muscle cells in vitro. ECM proteins can also be used to support 3D cell culture in vitro for modeling tumor development. ECM proteins play important roles in cell surface interactions. Cell adhesion can occur in two ways; by focal adhesions, connecting the ECM to actin filaments of the cell, and hemidesmosomes, connecting the ECM to intermediate filaments such as keratin. This cell-to-ECM adhesion is regulated by specific cell surface cellular adhesion molecules known as integrins. Integrins are cell surface proteins that bind cells to ECM structures, such as fibronectin and laminin, and also to integrin proteins on the surface of other cells. Fibronectin bind to ECM macromolecules and facilitate their binding to transmembrane integrins. The attachment of fibronectin to the extracellular domain initiates intracellular signaling pathways as well as association with the cellular cytoskeleton via a set of adaptor molecules such as actin.

Long-term in vitro culture of undifferentiated human embryonic stem cells (hESCs) traditionally requires a fibroblast feeder cell layer. Using feeder cells in hESC culture is highly laborious and limits large-scale hESC production for potential application in regenerative medicine. Replacing feeder cells with defined human extracellular matrix components or synthetic biomaterials would be ideal for large-scale production of clinical-grade hESCs.

Md Amranul H. et al. investigated the synthetic extracellular matrices to support improved cell attachment, propagation, differentiation and migration [68]. In general, ECM proteins from natural sources (e.g. collagen, laminin, or fibronectin) are advantageous for cell culture because of the presence of cell recognizable receptors, known as integrin-dependent interaction. However, complexities associated with natural materials, including complex structural composition, purification, immunogenicity and pathogen transmission have driven the development of synthetic

biomaterials for use as 2D or 3D extracellular microenvironments to mimic the regulatory characteristics of natural ECMs and ECM-bound growth factors [69]. The designing of artificial ECM should enable more efficient and scalable culture of ES cells, as well as greater control over material properties and tissue response.

*In vitro* with animal serum-free system, several researches investigated the used of silk sericin protein. Silk derived from silkworm *Bombyx mori* is a natural protein that is mainly made of sericin and fibroin proteins. Sericin constitutes 25-30% of silk protein and it envelops the fibroin fiber with successive sticky layers that help in the formation of a cocoon [70]. At present, sericin is mostly discarded in silk processing wastewater. The cocoon production is about 1 million tons (fresh weight) worldwide and this is equivalent to 400,000 tons of dry cocoon. Processing of this raw silk produces about 50,000 tons of sericin. If this sericin protein is recovered and recycles, it can represent a significant economic and social benefit.

Like fibroin, sericin is a macromolecular protein. Its molecular weight ranges widely from about 10 to over 300 *kDa*. The sericin protein is made of 18 amino acids most of which have strongly polar side groups such as hydroxyl, carboxyl, and amino groups. In addition, the amino acids serine and aspartic acid constitute approximately 33.4 % and 16.7 % of sericin, respectively. Sericin is a water-soluble protein. The small sericin peptides are soluble in cold water and can be recovered at early stages of raw silk production. The large sericin peptides are soluble in hot water and can be obtained at the latter stages of silk processing or from processes for silk degumming. Because of its properties, sericin is particularly used in food, cosmetics and pharmaceutical products as well as for biomaterials manufacture.

In the present study, there are reported that sericin induces the proliferation of several cell lines, including hybridoma cells, and is a candidate for a supplement for serum-free medium. A membrane composed of sericin and fibroin is an effective substrate for the proliferation of adherent animal cells and can be used as a substitute for collagen. Minoura et al. (1995) [71] and Tsukada et al. (1999) [72] investigated the attachment and growth of animal cells on films made of sericin and fibroin. Cell attachment and growth were dependent on maintaining a minimum of around 90% sericin in the composite membrane. Films of pure component proteins (i.e., fibroin and sericin) permitted cell attachment and growth comparable to that on collagen, a widely used substrate for mammalian cell culture.

In addition, plasma treatment process can be achieved the antimicrobial property of fluorine-containing plasma treated surfaces [73]. Moreover, the long term stability of coating antibacterial agent on the plasma treated surface can prevent the initial adhesion of bacteria to the surface and can kill them [74, 75]. Plasma sterilization is an alternative to other conventional sterilization methods like high temperature

sterilization, ethylene oxide sterilization and sterilization by radiation, especially for treatment of heat-sensitive materials. It makes the destruction of the microorganism cell wall which is the major mechanism of disinfection. Various treatment times were found to be effective for various microorganisms. Polymer sterilized by plasma showed little or no change in their 3D morphology, molecular weight or mechanical properties.

### **1.6 Stem Cells in Regenerative Medicine**

Stem cells have the remarkable potential to develop into many different cell types in the body during early life and growth. Stem cells are important for living organisms for many reasons. In some adult tissues, such as bone marrow, muscle and brain, discrete populations of adult stem cells generate replacements for cells that are lost through normal wear and tear, injury, or disease. Given their unique regenerative abilities, stem cells offer new potentials for treating diseases such as diabetes, and heart disease [76]. However, much work remains to be done in the laboratory and the clinic to understand how to use these cells for cell-based therapies to treat disease, which is referred to as regenerative or reparative medicine. There are many ways in which human stem cells can be used in research and the clinic. Studies of human embryonic stem cells (hESCs) have to concern the ethics of stem cell research [77, 78]. A primary research of human stem cells is start with the used of mesenchymal stem cells (MSCs). This can be achieved by the transplantation of cells, in sufficiently high numbers and quality, to a damaged target organ. MSCs from different sources, such as bone marrow cavity, so called BM-MSCs, umbilical cord blood, CBMSCs, or umbilical cord Wharton's jelly, WJMSCs have been identified as a highly potential of similar differentiation [79, 80]. However, several reports using these MSCs mentioned their poor adhesive capability or reduced attachment capacity following culture expansion on the available commercialized culture vessels [81, 82].

Long-term *in vitro* culture of undifferentiated stem cells traditionally requires a fibroblast feeder cell layer for hESCs and animal serum such as fetal bovine serum (FBS) for hMSCs. Using feeder cells or animal serum in culture system is highly laborious and limits large-scale production for potential application in regenerative medicine. Replacing feeder cells with defined human extracellular matrix (ECM) components or synthetic biomaterials would be ideal for large-scale production of clinical-grade hESCs and using serum-free condition such as natural silk protein would be ideal for clinical-grade hMSCs.

Extracellular matrix (ECM) have been found to cause regrowth and healing of tissue. In human fetuses, for example, the extracellular matrix works with stem cells to grow and regrow all parts of the human body, and fetuses can regrow anything that

gets damaged in the womb. ECM proteins are commonly used in cell culture systems to maintain stem and precursor cells in undifferentiated state during cell culture and function to induce differentiation of epithelial, endothelial and smooth muscle cells *in vitro*. However, controlling stem cell proliferation and differentiation using matrices from natural sources is still challenging due to complex and heterogeneous culture conditions. Moreover, the systematic investigation of the regulation of self-renewal and differentiation to lineage specific cells depends on the use of defined and stress-free culture conditions. Both goals can be achieved by the development of biomaterial design targeting ECM or growth factors for stem cell culture.

The designing of artificial biomaterial used as cell culture substrate should enable more efficient and scalable culture of stem cells, as well as greater control over material properties and tissue response.

### **1.6.1 Cell-Biomaterial Surface Interaction**

The cell adhesion is mediated by interacting between specific receptors on the cell surface and extracellular matrix (ECM). A protein layer of extracellular matrix (ECM) is able to interact specifically with integrin receptors of the cell surface. Thus, the polymer substrates coated with natural ECM proteins, such as collagen, fibronectin, hyaluronic acid and laminin, have been used for cell culture applications. The use of natural proteins, however, bears some disadvantages in the view of medical applications due to some risks such as undesirable infection. In addition, the activity of the proteins coated on the substrates is influenced by the conformation and/or orientation.

Cell interactions with polymers are usually studied using cell culture techniques. To study cell interactions, cells in culture are usually plated over a polymer surface and the extent of cell adhesion and spreading on the surface is measured. By maintaining the culture for longer periods, the influence of the substrate on cell viability, function, and motility can also be determined. The different between experimental techniques are potentially important for interpretation of interactions.

When a cell approaches a surface, most is anchorage dependent and require attachment to a solid surface for viability and growth. In tissue engineering, cell adhesion to a surface is critical because adhesion precedes other events, such as cell spreading, cell migration, and often differentiated cell function.

A variety of different techniques to quantify the extent and strength of cell adhesion and so many different techniques are usually difficult to compare studies performed by different investigators. However, the simplest methods involve three steps: (1) suspension of cells over a surface, (2) incubation of the dedimented cells in

culture medium for some period of time, and (3) detachment of non adherent cells under controlled conditions. The extent of cell adhesion is determined by measured either the number of cells that remain associated with the surface (the “adherent” cells) or the number of cells that were extracted with the washes. Radiolabeled or fluorescently labeled cells can be used to permit measurement of the number of attached cells. Alternatively, the number of attached cells can be determined by direct visualization, by measurement of the concentration of an intracellular enzyme, or by binding of a dye to an intracellular component such as DNA. In many cases, the adherent cells are further categorized based on morphological differences (e.g., extent of spreading, formation of actin filament bundles, presence of focal contacts). This technique is simple, rapid, and because it requires simple equipment, it is commonly performed.

Cell attachment, migration and growth on polymer surfaces appear to be mediated by proteins, either adsorbed from the culture medium or secreted by the cultured cells. Because it is difficult to study these effects *in situ* during cell culture, often the polymer surfaces are pretreated with purified protein solutions. In this way, the investigators hope that subsequent cell behavior on the surface will represent cell behavior in the presence of a stable layer of surface-bound protein.

### 1.6.2 Substrate Surface Properties

The extent and strength of cell adhesion, and subsequent cell proliferation and differentiation, depend strongly on the physical and chemical properties of the biomaterial surface, e.g. its chemical composition, energy, polarity and wettability. The chemical composition of the material surface is effect on the surface energy, polarity, wettability and zeta potential, and consequently the character of the cell-material interaction. For example, the presence of oxygen-containing chemical functional groups increases the energy, polarity and wettability of the material surface, and supports the adhesion and growth of cells on this surface [83, 84].

In the physics of solids, surface energy quantifies the disruption of the intermolecular bonds that occur when a surface is created. The material surface energy can be calculated from the contact angle measured between the material surface and liquids of various polarity, e.g. benzyl alcohol, diiodomethane, glycerol or water [85]. Polar liquids are well spread on polar surfaces, i.e. they have a low contact angle. A low contact angle between the material and water indicate good spreading of water on the material surface, i.e. hydrophilicity of the material, while a high water contact angle is a sign of the hydrophobicity of the material surface. Thus, hydrophilic surfaces are characterized by a high polar component of the surface energy.

The material surface energy including the nonpolar and polar molecules, the interactions of the former comes from the London forces. These forces are part of the van der Waals forces and represent the weak intermolecular forces. These forces can therefore act between molecules without permanent multipole moments. London forces are exhibited by nonpolar molecules because of the correlated movements of the electrons in interacting molecules. The polar component of surface energy comprises all other interactions due to non-London forces. Polar molecules interact through dipole-dipole intermolecular forces and hydrogen bonds. Molecular polarity is dependent on the difference in electronegativity between the atoms in a compound and the asymmetry of the structure of the compound. For example, a molecule of water is polar because of the unequal sharing of its electrons in a “bent” structure, whereas methane is considered nonpolar because the carbon shares the electrons with the hydrogen atoms almost uniformly.

On wettable surfaces, these molecules are adsorbed in a more flexible form, which allows them to be reorganized by the cells and thus provides access for cell adhesion receptors to adhesion motifs on these molecules. Cells adhered in higher numbers to more hydrophilic materials and were spread over a larger area. If the material is too hydrophobic, these molecules are adsorbed in a denatured and rigid state. Their geometrical conformation is inappropriate for binding to cells, because specific sites on these molecules (e.g. RGD-containing oligopeptides) are less accessible to cell adhesion receptors, e.g. integrins.

However, an extremely hydrophilic surface is not suitable for cell adsorption. The adsorption of a cell adhesion-mediating protein to an extremely hydrophilic surface does not allow protein adsorption at all, or the adsorption is weak and unstable, thus the proteins cannot provide an adequately firm anchor for the adhering cells. Although the adhesion ligands in the protein molecule are accessible for cell adhesion receptors, these receptors cannot form focal adhesion plaques and associate with paxillin, talin, vinculin, alpha-actinin and actin.

The chemical composition of the material surface is an important factor determining the surface energy, polarity, wettability and zeta potential, and consequently the character of the cell-material interaction. For example, oxygen-containing groups increase the polar component of the surface free energy of the polymer surface, making this surface more wettable, stickier and more susceptible to adsorption of adhesion-mediating ECM proteins, e.g. vitronectin, fibronectin, collagen or laminin. At the same time, the adsorption of cell non-adhesive molecules, e.g. albumin, is moderated because these molecules prefer to bind to less oxygenated and more hydrophobic surfaces. In the case of nitrogen-containing groups, it is well known that the hydrophilic and protein-containing surfaces are good for cell growth due to a positively charged amine group ( $-NH_2$ ) is highly reactive and therefore

believed to covalently couple with cell surface adhesive-proteins in aqueous environments [86, 87].

On functionalized self-assembled monolayers, the amount of adsorbed fibronectin decreased in the following order of functionalities:  $NH_2 > CH_3 > COOH > OH$ , while the adhesion of MC3T3-E1 osteoblast-like cells, mediated by  $\alpha_5\beta_1$  integrin adhesion receptors, increased in a similar order, i.e.  $CH_3 < NH_2 = COOH < OH$ , which can be explained by changes in the geometrical conformation of fibronectin [88].

The electrical charge of the material surfaces are also important factors for its colonization with cells. It has been shown repeatedly that there is better cell adhesion to positively charged surfaces than to negatively charged surfaces. The reason is that cell adhesion-mediating ECM molecules are negatively charged, thus they adsorb preferentially to positively charged surfaces (as described above for  $-NH_2$ ).

Like on surfaces with different wettability, also on surfaces with a different electrical charge, the spatial orientation and the biofunctionality of the adsorbed cell adhesion-promoting ECM proteins is more important than the absolute number of these molecules. Liu et al. [89] reported the amount of ECM protein osteopontin adsorbed to both surface of positively charged  $-NH_2$  group and negatively charged  $-COOH$  group was similar, but there is a much higher number and a much greater spreading area of bovine aortic endothelial cells on the  $-NH_2$  surface than on the  $-COOH$  surface. These results suggested that the orientation and the geometrical conformation of osteopontin was more favorable for cell adhesion and spreading on a positively charged  $-NH_2$  surface than on a negatively charged  $-COOH$  surface.

Surface microroughness is a more controversial factor affecting the behavior of cells on artificial materials. The cells typically studied on these materials, i.e. anchorage-dependent mammalian cells of various tissues and organs, including vascular tissue or bone, are usually between  $10 \mu m$  and  $50 \mu m$  in diameter, if they are in suspension, where they acquire a rounded shape. When adhered and spread on the material surface, their spreading area can reach from several hundreds to several thousands of  $\mu m^2$ . Thus, these cells are inherently sensitive to the microtopography of their environment, and many studies have reported that microroughness significantly affected the cell response to the material. Some studies have reported a positive influence of nanoscale roughness of the material surface has been unambiguously considered as a desirable factor that has a positive influence on the adhesion, growth and maturation of cells. The reason is that the nanostructure of a material resembles the nanoarchitecture of the natural ECM, e.g. its organization into nanofibers, nanocrystals, nanosized folds of ECM molecules, etc. On nanostructured surfaces, the cell adhesion-mediating ECM molecules therefore adsorb in an appropriate geometrical orientation which gives cell adhesion receptors access to

specific sites in ECM molecules, such as amino acid sequences like Arg-Gly-Asp (RGD), which serve as ligands for these receptors [90, 91]. From this point of view, surface nanoroughness can be considered to act synergetically with the moderate hydrophilicity of the material surface described above. This also promotes the adsorption of cell adhesion-mediating molecules in bioactive physiological conformations. One of these factors can enhance or even compensate the effect of the other factors. On surfaces with the same nanoscale roughness but different wettability, the cell colonization and function was higher on more wettable surfaces. Conversely, on surfaces of the same wettability, the cell performance was better on nanostructured surfaces.

For cells attached to a solid substrate, cell behavior and function depend on the characteristics of the substrate. Polymers can frequently be made more suitable for cell attachment and growth by surface modification. In fact, polystyrene (PS) substrates used for tissue culture are usually treated by glow discharge [92] or exposure to sulfuric acid to increase the number of charged groups at the surface, which improves attachment and growth of many types of cells. Many of the effects of surface modification appear to be secondary to increased adsorption of cell attachment proteins, such as fibronectin and vitronectin, to the surface.

### 1.7 Post-plasma Surface Process

A major concern for the practical relevance of a surface functionalization process is the durability of the product. Plasma modification processes of polymers generally leave the surface in an activated state. This causes a number of effects which are known to deteriorate the quality of a functionalization are called aging effect. The major post-plasma processes are *motility of surface functional groups, oxidation of functional groups and the role of dangling bonds*. An important question in contemporary polymer functionalization is how to overcome the negative effects of post-plasma reactions. While the deterioration of steep functionalization gradient by diffusion is difficult to tackle, for the treatment of dangling bonds two approaches exist. They can either be saturated by crosslinking of the polymer itself, or by attaching auxiliary substrates like radical scavengers or monomers.

#### *Motility of Surface Functional Groups*

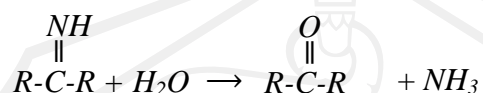
Plasma modification generally creates functional groups only in the very first surface layer(s). The resulting steep concentration gradients drives diffusion processes. Any polarity of functional groups enhances the energy density of the surface and exerts an additional driving force. Migration or rotation of functional groups into the surface has been observed on storage. They reduce the density of groups available at the surface. Foerch et al. [93], studied these effects by means of



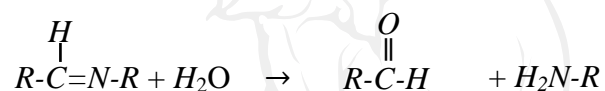
XPS and contact angle measurements. In crosslinked polymer surfaces the polymer chains form a tight network showed a reduced motility of surface functional groups.

#### *Oxidation of Functional Groups*

On contact to oxygen from air amino groups can oxidize to amides. These processes may also be efficiently initiated by peroxides formed in oxidation of dangling bonds. Another oxidation schemes was suggested by Foerch et al. [93] who assumed hydrolysis of primary imines by atmospheric water to ketones



to be responsible for fast post-process oxygen incorporation accompanied by loss of nitrogen. The fragmentating hydrolysis of secondary imines to a carbonyl- and a primary-amino-terminated polymer was supported to be slower.



#### *The Role of Dangling Bonds*

Crosslinking is a way of de-activating dangling bonds. It stabilizes the polymer surface against autoxidation and reduction in molecular weight. Unsaturated and aromatic polymers have a higher propensity of crosslinking than aliphatic ones. The impact of ions from an inert gas discharge to provide kinetic energy for breaking polymer chains creates chain scission. Polymers may show spontaneous crosslinking of polymer chains after chain scission. Crosslinking creates a polymer network. The propensity to crosslinking is enhanced for unsaturated and aromatic hydrocarbons. Helium, the lightest gas, was found to be suited best for crosslinking the outermost polymer surface by Clark and Dilks [94] due to its low kinetic impact on the surface.

Autoxidation is the mechanism behind post-process oxidation observed after plasma modifications of polymers. It promotes polymer degradation and largely enhances the oxygen content of the surface. Dangling bonds can react with molecular oxygen from the atmosphere. The reaction occurs already at low temperatures and leads to formation of peroxides.

### *Hydrophobic/Hydrophilic Recovery*

Hydrophobic recovery and loss of adhesive properties can occur in plasma-treated polymers that are stored in ambient air for extended period of time [95-97]. Hydrophobic recovery is an indication of polymer surface instability in which the hydrophilicity decreases with time stored in ambient air due to surface configuration changes. A plasma-treated polymer that undergoes aging effects could be useless for the desired application once the hydrophobic recovery is significant.

The functional groups generated on the polymer surface by plasma treatment are not stable and have higher mobility than in polymer bulk. In order to become stable after plasma treatment, the surface is likely to be reoriented by the migration of short chain oxidized molecules and the diffusion of oxidized functional groups onto polymer bulk.

Aging studies of plasma polymers are important, as marked change to the surface chemistry of the polymers may happen over the storage period, but such effects have often been overlooked or not recognized. In the absence of characterization of aging, erroneous conclusions can be drawn on relationships between surface chemical compositions and biological responses, for example, if cell attachment and XPS analyses are performed after different times of aging.

Plasma-treated polymer surfaces and plasma polymers have often been observed to undergo substantial changes in their surface chemical compositions and properties, such as contact angles (CAs), with time as they are stored. This 'aging' is usually interpreted in terms of two fundamental processes: post-plasma oxidation, initiated by reaction between remaining radicals and in-diffusing atmospheric oxygen, and surface adaptation, which is a consequence of reputation motions that move some of the polymer chains from the surface into the bulk.

### **1.8 The Interaction of Water and Material Surface**

To evaluate the hydrophobic-hydrophilic character and the wettability of a surface have been characterized macroscopically by the contact angle at the interfaces. Wetting is the contact between a liquid and a solid surface, resulting from intermolecular interactions when the two are brought together. The amount of wetting depends on the energies (or surface tensions) of the interfaces involved such that the total energy is minimized. The degree of wetting is described by the contact angle ( $\theta$ ) [98]. The contact angle is a measure of the ability of a liquid to spread on the outline tangent of a drop deposited on a solid and the surface of this solid. The contact angle is linked to the surface energy and so one can calculate the surface energy and discriminate between polar and apolar interactions. When the liquid is

water and it spreads over the surface, the surface is said to be hydrophilic and the contact angle range from  $0^\circ$  to  $30^\circ$  (or  $< 90^\circ$ ). If the solid surface is hydrophobic, the contact angle will be larger than  $90^\circ$ , as shown in Figure 1.7 [99].

In particular, hydrophilization of polymer by oxygen or nitrogen plasmas has found wide use. In biomedical applications, plasma hydrophilization can improve the biocompatibility of polymers as well as affect the attachment density of cells [100, 101] and the adsorption of proteins [102].

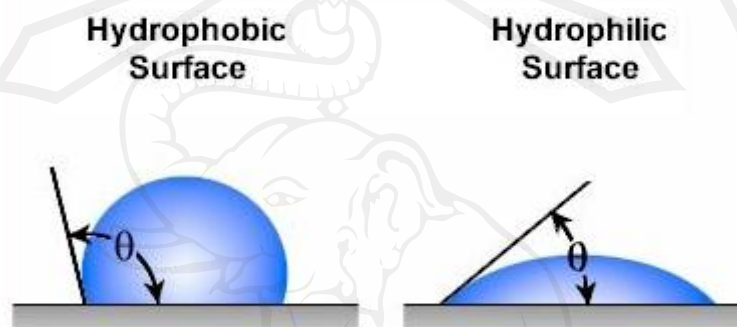


Figure 1.7 The contact angle of hydrophobic and hydrophilic surfaces.

### 1.9 Review of Literatures

Many research groups have been studied and reported the various effects of surface modification methods to improve the surface properties of biomedical materials used in biological system applications, especially in cell therapy development. Several studies reported the used of plasma nanotechnology successful approaches to improve the biocompatibility of polymer surfaces which were used in cell culture system to enhance in sufficient cell proliferation, expand cell adhesion and increase cell yields. Due to most of the commercialized designed surfaces are well developed for common types of cells in culture, but more specific cell types require more advanced modifications. In cell therapy, MSCs from different sources, such as bone marrow cavity, so called BM-MSCs, umbilical cord blood, CBMSCs, or umbilical cord Wharton's jelly have been used to treat human disease [103, 104]. However, several reports using these MSCs mentioned their poor adhesive capability or reduced attachment capacity following culture expansion on the available commercialized culture vessels [105]. Polystyrene (PS) has been used since 1965 [106] as a popular culture vessels for microbes due to its excellent durability, good optical and non-toxicity. PS itself is unsuitable for eukaryotic cell culture, because of its hydrophobic nature. Therefore, surface treatments are required to optimize cell adhesion [107].

It is well known that the hydrophilic and protein-containing (extracellular matrix molecules) surfaces are known to be good for cell growth. Due to the amine group ( $-NH_2$ ), a positively charged, is highly reactive and therefore believed to covalently couple proteins in aqueous environments [108]. A number of studies have shown plasma deposited films can lead to enhanced levels of cell attachment, which have attributed to the presence of various functional groups including: hydroxyl [109], carbonyl [110], carboxyl [111], and amine [112, 113].

Kuzuya et al. [112] reported a novel method for fabricating a durable hydrophilic surface on hydrophobic polymer materials, modified by plasma treatment. They showed that plasma assisted immobilization of vinylmethylether-maleic anhydride copolymer (VEMA) onto PE surface. It showed that effective durability of wettability, for a long period of time is based on the water contact angle measurement. Including, Sasai Y. et al. [111] immobilized of VEMA on PS by plasma-induced crosslink reaction to introduce a large amount of carboxylic group onto PS surface. The PS/VEMAC surface thus fabricated exhibited a durable hydrophilicity, a good LNCap cell adhesion and spreading property as compared with non-treated PS.

According to Yang et al. [114], amine functional groups were grafted onto polymethylene terephthalate (PET) surfaces by dielectric barrier discharge (DBD) plasma, with allylamine as monomer. It shown that plasma treated PET films had efficient cell adsorption behaviour and the high rate of cell proliferation was visualized.

As Salerno et al. [115] reported PEEK-WC-PU membranes were modified with an  $NH_3$  glow discharge process to graft N-containing functional groups at their surface, in order to improve the maintenance of human hepatocytes. They found that N-containing groups grafted to the surface of the membranes improved the initial steps of adhesion and the maintenance of the phenotype and differentiated functions of cells.

Finke et al. [116] studied the effect of positively charged plasma polymerization on initial osteoblastic focal adhesion on titanium surfaces. They found that allylamine plasma polymer layer (PPAAm) of titanium, with a high density of positively charged amino groups, is advantageous concerning osteoblastic focal adhesion as vinculin and paxillin, actin cytoskeleton development, and in consequence in differentiated cell functions, compared to a pure titanium surface.

The novel method of introducing a durable surface wettability and minimizing its decay with time on several hydrophobic polymers is a matter of great concern in the practical use. It is well known that the use of an inert gas discharge to minimized the ageing phenomenon by stabilizing the surface layer via crosslinking [117, 118]. Kuzuya et al. [112, 118] reported that the argon plasma irradiation to PS generates the highly crosslinked layer on the PS surface because cyclohexadienyl-type radicals formed in plasma-irradiated PS are greatly implicated in crosslinking. In addition to this, Liu et al. [119] investigated the enhancement of the molecular nitrogen dissociation and ionization levels by argon mixture in flue nitrogen plasma. They found that the dissociation rate  $[N]$  of  $N_2$  molecules was enhanced as the mixture

quantity of Ar increased from  $0.06 \text{ m}^3/\text{h}$  to  $0.9 \text{ m}^3/\text{h}$ . Naveed et al. [120] reported the effects of helium gas mixing on the production of active species in nitrogen plasma. They found that the electron temperature could be increased dramatically by mixing helium in to the nitrogen plasma, which plays a significant part in increasing the intensity of active species through the Penning effect of metastable states of the helium.

Generally, *In vitro* mammalian cell culture, mammal-derived factors including fetal bovine serum (FBS) are often used as growth factors into the media and are cause the concern about the risk of disease transmission such as abnormal [121, 122]. Therefore, serum- and mammal-free culture is strongly required. We focused on sericin hydrolysates, which is a protein was extracted from the glue of cocoons, is the alternative strategy used as supplemented into the culture media. Silk proteins are added to culture media used as serum free media for cell culture, additionally, are coated onto Petri dishes enhance attachment of cultured human skin fibroblasts [123]. In comparison with bovine serum albumin (BSA), sericin had an equivalent effect on the proliferation of the hybridomas with BSA and the activity of sericin was not affected by autoclaving [122]. Commonly, sericin protein absorbed onto various substrates spontaneously, however, sericin itself water soluble, in the case of cell culture application, their immediately removed from the surface when exposed to the culture media and could not support the cell growth for a prolonged time.

Amorphous carbon (a-C) is now being attractive in biological applications because it can be prepared relatively inexpensively for a wide variety of low-cost precursors, it is typically biocompatible and quite chemically stable under nonoxidizing conditions [124-126]. Of prominent features of the carbon film including as a high specific-area as well, porous carbon is more binding active molecule and more resistant to structural changes by hydrolytic effects in aqueous environments.

It is well known that attachment in the first stage is controlled by the interaction between cell and surface materials, meanwhile, the longer term adhesion and proliferation are associated to the presence of specific biological molecules and/or proteins [127]. Mechanism of immobilization biomolecules by covalent bond offers several advantages by providing the most stable bond between the biomolecule and the functionalized polymer surface. The surface-immobilized biomolecule with chemical bond could lead to permanent or long-term retention. For covalent binding to an inert solid polymer surface, the surface must first be modified to provide reactive groups (e.g.  $-\text{OH}$ ,  $-\text{NH}_2$ ,  $-\text{COOH}$ ,  $-\text{SH}$  or  $-\text{CH}=\text{CH}_2$ ) for the subsequent immobilization step [127, 128]. In the case of cell culture applications, a covalent linkage ensure that the bioactive compound will not suddenly removed from surface when exposed to the culture media or migrate to the culture media for a period of a long time.

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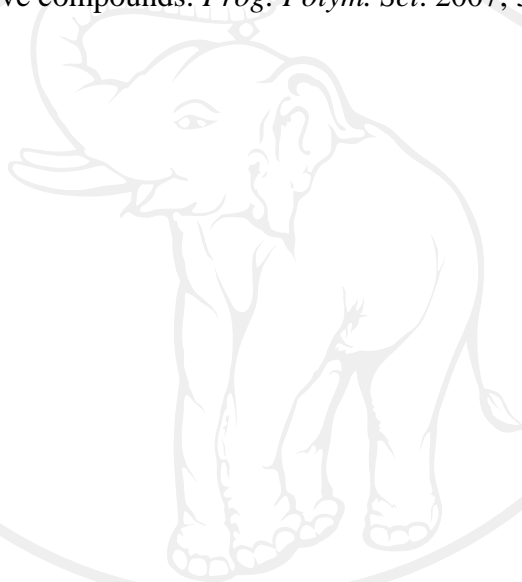
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